STUDIES OF THE METABOLISM OF PHOSPHORUS IN THE DEVELOPMENT OF THE SEA URCHIN, STRONGYLOCENTROTUS PURPURATUS [1]

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The eggs of sea urchins contain a group of acid-soluble phosphorylated compounds whose barium salts are soluble in alcohol. The magnitude of this fraction is unusually large in comparison with other animal tissues. The unfertilized eggs of various sea urchins and asteroids have been reported to have from 9.3% (Mende and Chambers, 1953) to 39.7% (Whiteley, 1949) of their acid-soluble phosphorus in the form of barium-soluble, alcohol-soluble compounds. In various vertebrate tissues these compounds comprise about 1 to 8.3% of the acid-soluble phosphorus (LePage, 1948; Sacks, 1949). Aside from quantitative measurements, very little is known about these compounds or the part they play in the metabolism of the echinoderm egg. Lindberg (1943) reported finding a compound in this fraction from the egg of the heart urchin, *Brissopsis*, which he subsequently (1946) identified as 1, 2-propanediol phosphate, whose metabolism he studied. Hörstadius and Gustafson (1947) reported some animalizing effect by both synthetic propanediol phosphate and a natural compound, and Borei (1948) has described the effect of this ester on egg respiration. However, Rudney (1952, 1954) has reported that the barium salt of propanediol phosphate is not soluble in alcohol, and the significance of the above observations, therefore, is not clear. Mende and Chambers (1953) have shown that some of the phosphorus of the barium-soluble, alcohol-soluble fraction of *Asterias forbesii* and *Strongylocentrotus dröbachiensis* is acid-labile.

This paper supplies information relative to the extent to which the barium-soluble, alcohol-soluble fraction serves as a storage material for metabolism, and the rate of turnover of the compounds of this fraction in the eggs and embryos of the sea urchin, *Strongylocentrotus purpuratus*. The possibility has been explored that there are special periods in the developmental process when the metabolic activity of these compounds varies. The existence of propanediol phosphate as a component of this fraction has been examined, and preliminary chromatographic determination of the number of esters in the fraction has been made. As an outgrowth of the turnover studies, an unusual pattern of permeability of embryos to inorganic phosphate during larval development has been found.

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Materials and Methods

Embryological. The sea urchins used in these experiments were *Strongylocentrotus purpuratus* (Stimpson), collected from San Juan Island, Washington. Animals were induced to spawn by the injection of isotonic KCl (Tyler, 1949). Batches of eggs less than 95% fertilizable were not used. "Dry" sperm were diluted to a 1% suspension immediately before use. After insemination, excess sperm were removed from eggs by washing with fresh sea water. Filtered sea water was used throughout.

In experiments on embryos older than the two-cell stage suspensions of developing embryos were cultured in a four-liter Erlenmeyer flask which lay at an angle of about 30° in a water bath held to 11.0 ± 0.1° C. by a thermostat. Egg suspensions, ranging in concentration from 0.3 to 0.9% by volume, were gently agitated by rotating the flask at 28 rpm. For egg counts, 10-ml. aliquots were taken and, after appropriate dilution, ten individual counts were made and averaged.

Aliquots of 300 ml. were taken from the stock suspensions at different stages of development for analysis of phosphates and for separate incubation with radioactive phosphate. Carrier-free radioactive phosphate was added to give a final radioactivity of 0.04 μc./ml., and incubation was continued in the same manner as described above, normally for 60 minutes. Duplicate 10-ml. aliquots were then taken for analysis of total phosphorus. The embryos were removed from the remainder of the suspension in a lucite centrifuge patterned after the Foerst plankton centrifuge. After washing with fresh sea water the embryos were frozen and stored for later analysis.

In experiments with eggs prior to the first cleavage the incubation times and radiophosphate concentrations varied and are given with the results. These samples were analyzed immediately without freezing.

Analytical. The extraction of the barium-soluble, alcohol-soluble material from the embryos followed the procedure of Sacks (1949) and that of Umbreit, Burris and Stauffer (1949). In some instances the other phosphate fractions identified below were also prepared. The sample was thawed and homogenized in a Potter-Elvehjem type of homogenizer in 2 ml. of 0.5 N $HClO_4$. This and the subsequent steps were carried out near 0° C. The homogenate was centrifuged and the residue (acid-insoluble fraction) re-homogenized twice with 1 ml. of 0.5 N $HClO_4$. The three supernatants were pooled, four volumes of re-distilled 95% ethyl alcohol added, and the mixture set aside for one hour. The extract was centrifuged, the residue (acid-soluble, alcohol-insoluble, probably polysaccharides) washed twice with acid-ethanol, the combined supernatants adjusted to pH 8.2, and 1 ml. of 25% barium acetate added. The precipitate (barium-insoluble plus barium-soluble, alcohol-insoluble fractions) that formed after one hour was centrifuged and washed with ethanol adjusted to pH 8.2. The supernatants were brought to 50.0 ml. This is the barium-soluble, alcohol-soluble fraction.

Phosphorus was determined by the method of Berenblum and Chain (1938), and, in a few cases, by a modified Fiske and SubbaRow (1925) method with ferrous sulfate as reducing agent. The dried samples, blanks, and standards were digested in 70% $HClO_4$. The blue isobutanol extracts resulting from the phosphorus determinations were used for radioactivity assay. Aliquots were pipetted into planchets, dried, and counted with a Geiger-Müller counter equipped with an end-window tube

with window thickness of 3.26 mg./cm.^{2} Samples were counted to an error of less than 1%. In experiments with pre-cleavage stages, where phosphate was analyzed by the Fiske and SubbaRow method, aliquots of eggs or extracts were dried directly on planchets.

Paper chromatographic analysis for phosphate esters was carried out using a chromatographic system the details of which will be published elsewhere. The solvent system contained n-butanol, n-heptyl amine, and water. The descending method was used with washed Whatman No. 1 filter paper. The chromatograms were run at 1° C. for about 10 hours and developed by spraying with the Hanes-Isherwood molybdate reagent, heating at 80° C. for 5 minutes (Hanes and Isherwood, 1949), and irradiating with ultraviolet light at wave-length 2537 Å (Bandurski and Axelrod, 1951).

Results

If the phosphorus-containing compounds of the barium-soluble, alcohol-soluble fraction serve as a reserve of phosphorus, energy, or precursors of other substances during development, an indication of this function would probably be given by a decrease in the concentration of the phosphorus in the fraction as development progresses. Two experiments, each involving the eggs of a single sea urchin, were carried out to determine if this occurs. In each a large batch of fertilized eggs was cultured at 11° C. to the early pluteus stage. Aliquots were taken at intervals for determination of total phosphorus and barium-soluble, alcohol-soluble phosphorus. The results are given in columns three and four of Table I, and are shown in

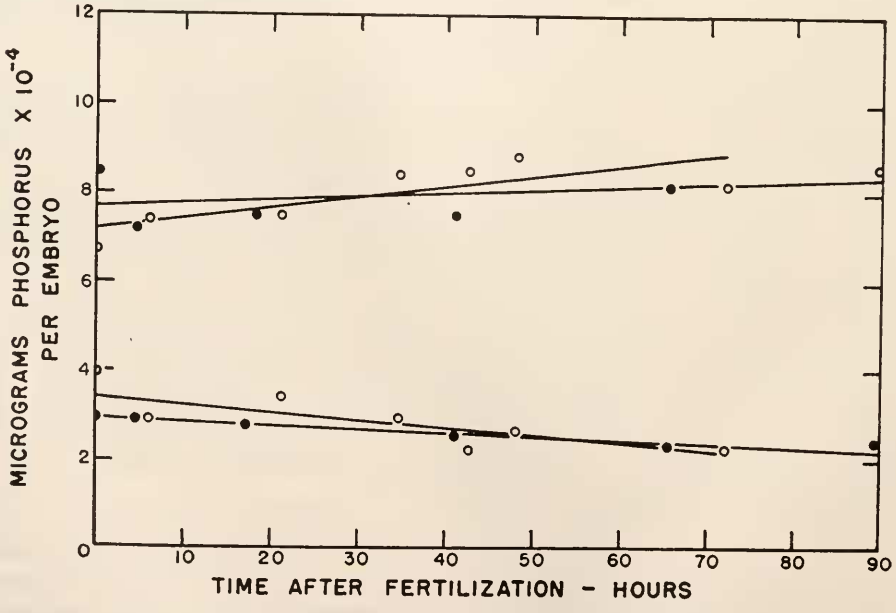

Figure 1. Total (upper curve) and barium-soluble, alcohol-soluble phosphorus content (lower curve) of developing embryos of *Strongylocentrotus purpuratus*. Solid circles are data from Experiment 1 and open circles are from Experiment 2 of Table I.

TABLE I

Amount of phosphorus and rates of incorporation of radioactive phosphorus in embryos and barium-soluble, alcohol-soluble compounds of embryos of the sea urchin, Strongylocentrotus purpuratus

Embryos		Phosphorus content μg./egg		Radioactive phosphorus content (cts./min./egg/hr.)		% BSAS-P^{32}	Specific activity (cts./min./μgP/hr.)		% BSAS-P^{32}
Age	Stage	BSAS-P	Total P	BSAS-P^{32}	Total P^{32}		BSAS-P^{32}	Total P^{32}	
				Experiment No. 1					
0 hrs.	UF*	3.0×10^{-4}	8.5×10^{-4}	—	—	—	—	—	—
4½	2-Cl	2.9	7.2	6.5×10^{-2}	120×10^{-2}	5.4	225	1710	13.2
17	UB	2.8	7.6	15.2	221	6.9	532	2910	18.3
41	EG	2.5	7.6	2.8**	36**	7.8	108**	472**	22.9
65½	LG	2.3	6.2	13.8	193	7.2	595	2240	26.6
89½	EP	2.4	8.6	6.4	140	4.6	262	1550	16.9
				Experiment No. 2					
0	UF	3.9	6.7	—	—	—	—	—	—
6	4-Cl	2.9	7.4	3.9	56	7.0	134	732	18.3
21	UB	3.4	7.6	13.3	241	5.4	384	3090	12.4
34½	EG	2.9	8.4	24.8	392	6.4	863	4520	19.1
42½	EG	2.2	8.5	19.0	332	5.7	868	3760	23.1
48	MG	2.6	8.8	17.3	248	7.0	657	3360	19.6
72	PR	2.2	8.2	7.2	141	5.1	324	1670	19.4
				Experiment No. 3					
43	EG	3.0	12.0	29.6	559	5.3	1120	5420	20.6

* Eggs from a single female used for each experiment. UF = unfertilized, 2-Cl = 2-cell stage, 4-Cl = 4-cell stage, UB = unhatched blastula, EG = early gastrula, MG = mid-gastrula, LG = late gastrula, PR = prism, EP = early pluteus.

** These data low, presumably by a factor of 10, due to a technical error. Experiment No. 3 was run at 43 hours to check this point.

Figure 1, the curves of which are fitted by the method of least squares. The quantity of barium-soluble, alcohol-soluble phosphorus decreases at a uniform rate throughout the non-feeding stages of larval development. The rate of utilization in Experiment 1 is 7.4×10^{-7} microgram/egg/hour and in Experiment 2 is 20×10^{-7} microgram/egg/hour. The per cent of the fraction present at the beginning of development utilized in reaching the prism stage (72 hours) is 18.5% and 40.7% for Experiments 1 and 2, respectively (based on the fitted curves). It seems safe to conclude that the utilization of the material contributed appreciably to the general metabolism since it involves mobilization on the average of 13.6% of the total phosphorus of the unfertilized egg. No significant variations in the rate of utilization correlated with visible aspects of morphogenesis are apparent in either experiment.

TABLE II

Distribution of radiophosphorus in barium-soluble, alcohol-soluble and other phosphorus-containing fractions in unfertilized and fertilized eggs. The per cent of radiophosphorus in each fraction is calculated with acid-insoluble plus acid-soluble equal to 100%

Fraction	Unfertilized eggs*			Fertilized eggs**		
	cts./min./ml.	Per cent P^{32}	Specific activity cts./min./μg P	cts./min./ml.	Per cent P^{32}	Specific activity cts./min./μg P
Total egg	—	—	—	188,500	100.3%	240
Acid-insoluble	24,050	29.2%	28.3	14,690	7.87	33.4
Acid-soluble	58,600	71.0	75.1	172,100	92.0	486
Barium-insoluble plus barium-soluble, alcohol-insoluble	60,100	73.0	95.5	160,500	85.9	596
Barium-soluble, alcohol-soluble	1,180	1.40	4.37	1,350	0.72	11.6

* Incubated 9 hours in P^{32} concentration of 0.04 μc/ml.
** Incubated one hour, before first cleavage, in P^{32} concentration of 0.02 μc/ml.

Among analyses done by both of the present authors, as well as those by others, the variation in the quantity of the barium-soluble, alcohol-soluble phosphorus is broad. Data of the present authors include values of 3.9, 3.0, 1.05 and 0.98×10^{-4} micrograms per egg, and many other analyses for which egg counts are not available indicate that the fraction from unfertilized eggs contains from 12.0% to 58% of the total phosphorus. Variations in multiplicate analyses rarely are of appreciable magnitude, usually amounting to only a few per cent; it is believed the large differences from batch to batch represent true biological variations. Sacks (1949) reported variations of comparable magnitude in this fraction extracted from rat liver. Mende and Chambers (1953) found different batches of eggs of *Asterias forbesii* to contain 322, 153, and 138 μg. P/ml., and *Strongylocentrotus dröbachiensis* to contain 59 and 44 μg. P/ml.

During the period of development covered in these experiments there was an increase in the total egg phosphorus. In Experiment 1 this amounted to an increase of 9.1%, and in Experiment 2 of 23%, as calculated from the fitted curves.

Although the barium-soluble, alcohol-soluble fraction shows an appreciable decrease during development, the possibility exists that there is a simultaneous synthesis of some of the compounds in the fraction. The question of synthesis was examined in unfertilized eggs and in embryos by adding radioactive inorganic phosphate to cultures and determining the radioactivity of the fraction and of the whole eggs after appropriate time intervals.

Unfertilized sea urchin eggs take up radioactive phosphate from sea water at an extremely low rate. To obtain sufficient activity in the phosphate fractions of such eggs, they were incubated for 9 hours at 12.0° C. in sea water containing 0.04 μc. radiophosphate/ml. The egg concentration was 0.4% by volume. Of a small sample inseminated after this incubation, more than 95% fertilized and developed to the early pluteus. The data in Table II show that the incorporation into the barium-

soluble, alcohol-soluble fraction prior to fertilization is very low; even after prolonged exposure to radiophosphate only 1.4% of the total activity of the egg was in this fraction. In a second experiment the figure was 0.78%. In confirmation of other investigators most of the activity enters the fraction containing inorganic phosphate, nucleotide phosphate, and labile esters, though in the 9-hour experiment very much more is found in the acid-insoluble fraction than has been reported before (Chambers and White, 1954).

The same low rate of incorporation of phosphate into this fraction persists directly after fertilization, before the first cleavage. This is apparent from the second experiment of Table II, in which a 1% suspension of eggs inseminated 12 minutes earlier was incubated for 60 minutes with 0.02 μc./ml. of radiophosphate. Although considerably more phosphate penetrated into these than into unfertilized eggs, the proportion in the barium-soluble, alcohol-soluble fraction is still only 0.7% of the whole. In comparable experiments, neither mono-iodoacetate nor 2,4-dinitrophenol, added with the radiophosphate, changed this pattern markedly, though both inhibited the uptake of phosphate by the egg.

The subsequent embryonic period was examined from the two-celled embryo to the early pluteus stage at 90 hours in the experiments of Table I. In these the time of exposure to radiophosphate at each stage examined was 60 minutes. Columns 5 through 10 of Table I present the pertinent determinations of radioactivities. Of the radiophosphorus that enters the embryos during one hour, an average of 6.5% (4.6 to 7.8%, Column 7) is incorporated into the fraction. There is no consistent pattern of change in this value during the rest of the development. Considered in terms of specific activities, the fraction attains a level that is about 19% (12.4% to 26.6%, Column 10) of the specific activity of the total egg phosphorus, again with no consistent change during the rest of the development. This contrasts with 5% for the freshly fertilized eggs in the experiment of Table II. Although the components of this fraction are metabolically very inactive in the unfertilized egg and before the first cleavage, it is concluded that one or two hours after fertilization at least part of the barium-soluble, alcohol-soluble fraction becomes moderately stimulated metabolically relative to phosphorus compounds of the egg as a whole, and this increased level of metabolic activity is maintained with approximate constancy until the pluteus stage.

The specific activities of the total egg phosphorus and of the barium-soluble, alcohol-soluble fraction are plotted against age of the embryos in Figure 2. It should be noted that these curves are not cumulative uptake curves, but rather are rate curves, each point representing the counts per minute per microgram of phosphorus per 60 minutes exposure at the particular age indicated. The rate of incorporation into the fraction is seen to increase to a maximum at about 40 hours, and then decline subsequent to this time. The peak of activity at 40 hours probably does not represent a special stimulation in metabolism within the fraction leading up to gastrulation, because, as was pointed out above, the activity of the fraction, when expressed as percentage of the whole embryo, is constant. Rather the peak reflects closely the uptake curve for the whole embryo, and is probably due to a change in permeability of the embryo to inorganic phosphate from the sea water.

An unanticipated finding in these experiments is that the rate of uptake in both the total phosphorus and the barium-soluble, alcohol-soluble fraction varies markedly in the different phases of development. The rate of uptake which begins to increase

15 or 20 minutes after fertilization (Abelson, 1947; Brooks and Chambers, 1948; Whiteley, 1949) continues to increase markedly until the stage of early gastrulation, at 34 to 43 hours in the different experiments. The rate then begins to decrease

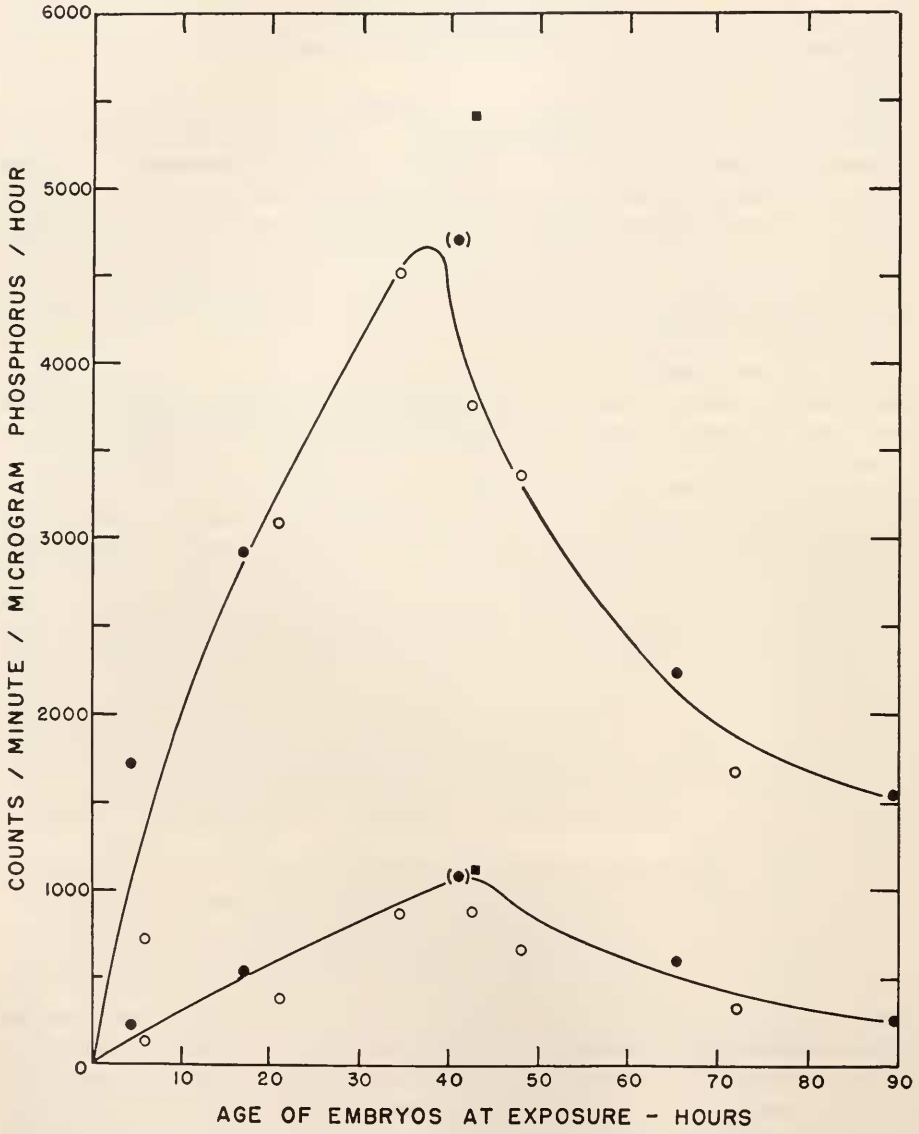

FIGURE 2. Rate of uptake of radiophosphate into the embryos of *Strongylocentrotus purpuratus* (upper curve) and into the barium-soluble, alcohol-soluble phosphate fraction (lower curve) of these embryos. Solid circles are data from Experiment 1, open circles are from Experiment 2, and squares from Experiment 3 of Table I. The bracketed solid circles at 41 hours have been multiplied by 10 (see footnote to Table I). Specific activities are in counts/min./μg. P/hour.

sharply, tending to level off in the prism and early pluteus stages. As nearly as could be determined, the inflection in the rate coincides with the onset of gastrulation. This pattern of uptake is shown when the data are considered either on the basis of counts per minute per egg or of specific activity.

This change of permeability to orthophosphate is not, however, universal for all phosphate compounds. The permeability of embryos to propanediol phosphate, determined in an experiment similar to those with orthophosphate but using labeled ester instead, reaches a maximum rate earlier in development (mid-blastula) and thereafter does not decrease through the pluteus stage. Even in the absence of information needed to calculate permeability constants for the ester and for orthophosphate, it appears that the ester penetrates much more slowly. Different mechanisms for the penetration of the two substances probably exist.

The composition of the barium-soluble, alcohol-soluble fraction in this material is unknown. Lindberg (1943) had reported the identification of 1,2-propanediol phosphate in this fraction in sea urchin eggs, but Rudney (1952, 1954) presents cogent reasons for believing this ester separates into the barium-soluble, alcohol-insoluble fraction in rat liver. Sea urchin eggs possess very large amounts of barium-soluble, alcohol-soluble phosphates and of polysaccharides. Interference by the polysaccharides with the clean separation of esters might account for different distribution of propanediol phosphate reported by Lindberg and Rudney. To determine the solubility of propanediol phosphate under the exact conditions used in this investigation, fractionations were made of sea urchin egg homogenates containing known amounts of synthetic 1,2-propanediol phosphate labeled with radioactive phosphorus.

Two separate lots of propanediol phosphate labeled with P^{32} were synthesized by the method described by Lampson and Lardy (1949), using 0.188 and 0.150 millicurie P^{32} in the reaction tubes for the two syntheses. The lead salts obtained were converted to the free acid with dilute sulfuric acid in the first case and H_2S in the other, and remaining traces of lead removed with Dowex-50 in the hydrogen form. The solution of lot 1 was neutralized to pH 7.0. This product contained no inorganic phosphate. The specific activity of the ester was 1439 counts per minute per microgram phosphorus under the standard counting conditions. The second lot, subjected to paper chromatographic analysis, showed a component with the same R_f as a commercial preparation (Nutritional Biochemicals Corp.) and a very faint unidentified second component with much less than 1% of the total activity. No inorganic phosphate was present. The specific activity was not determined.

In each of two separate experiments,[3] a mass of approximately a million unfertilized eggs having an estimated barium-soluble, alcohol-soluble phosphorus content of 300 micrograms was homogenized with cold 0.5 N $HClO_4$. Three hundred to 350 micrograms of phosphorus in the form of labeled propanediol phosphate were added to increase by a significant amount the content of supposed propanediol phosphate already in the eggs. The fractionation was completed in the usual manner and the radioactivity in the various fractions measured with the results given in Table III. Essentially the entire amount of radioactivity was found in the barium-insoluble, plus barium-soluble, alcohol-insoluble fraction. It is concluded, in agree-

[3] We are pleased to acknowledge the help of Miss Kathryn Eschenberg in one of these experiments.

ment with Rudney, that the barium-soluble, alcohol-soluble fraction does not contain propanediol phosphate. This leaves us with no specific information as to the composition of this very large fraction of these eggs.

A preliminary examination of the fraction has been made by paper chromatography. From a number of experiments in which, after isolation, it was subjected to various desalting pre-treatments, evidence for at least three components was derived. In all of these pre-treatments the samples were desalted with Dowex-50 in the hydrogen form and concentrated by evaporation. In some cases the phosphates precipitable by basic lead acetate were chromatographed, and in one the sample was treated with Dowex-2-OH. With different treatments, and depending on the

TABLE III

Recovery of phosphorus-labeled 1,2-propanediol phosphate when added to homogenates of unfertilized eggs in perchloric acid and subjected to barium and alcohol fractionation

Fraction	Experiment 1		Experiment 2	
	cts./min.	% of total activity	cts./min.	% of total activity
Acid-insoluble	31	0.3	38	0.4
Acid-soluble				
Alcohol-insoluble	104	1.0	60	0.7
Barium-insoluble and barium-soluble, alcohol-insoluble	10,203	96.4	8,825	97.8
Barium-soluble, alcohol-soluble	247	2.3	100	1.1
Totals	10,585*	100%	9,023	100%

* 11,174 counts were added to the homogenate. Recovery 94.6%.

amount of sample, one or two clear spots were detectable. Compounds with R_f's of 0.12, 0.29, and 0.71 were found. With this system of chromatography, inorganic phosphate has an R_f of 0.54 and propanediol phosphate, either commercial or prepared by us, has an R_f of 0.73. The similarity between this R_f and that of the unknown at 0.71 is not taken as evidence of identity because other substances, for example glycerol phosphate, have the same R_f in this system.

DISCUSSION

The results of the present investigation indicate that there are at least three components in the barium-soluble, alcohol-soluble fraction. From its magnitude it is possible that one of these could serve as a storage compound of some kind, and the gradual, uniform utilization of the material during development would be in accord with this. The experiments with radioactive orthophosphate demonstrate an appreciable, though not great, synthesis in the fraction, probably in one or more components other than the storage ones.

This synthesis is extremely low prior to fertilization and in the first hour thereafter, but increases demonstrably beginning with the first cleavage. Cleavage initiates an increase in activity in this fraction that is greater than the average increase

for the phosphorus compounds of the egg; before the first cleavage the specific activity in the fraction is 5% of that in the total egg phosphorus, while in subsequent stages it is about 19% of the total. The percentage of radioactive phosphorus that is incorporated into the fraction in embryos of different stages of development after cleavage starts is rather constant, despite large differences in total amount of radiophosphorus that enters the embryo. This suggests that the limiting factor for the synthesis of the component is the availability of phosphate rather than the level of activity of the synthesizing enzymes. Chambers and White (1949) supply evidence that the eggs of this species have a very small inorganic phosphate pool, especially after fertilization, which would be in accord with this idea. The constancy indicates that there are no special periods during development after cleavage in which turnover in this fraction is especially rapid relative to the turnover of the other phosphorus compounds of the embryo.

The evidence used by Lindberg (1946) for identification of propanediol phosphate isolated from cow brain is extensive, but it is not clear in his 1943 or 1946 papers on what basis propanediol phosphate is considered to be a component of the barium-soluble, alcohol-soluble fraction, other than that the fraction has a high stability toward acid and alkaline hydrolysis. His identification of the ester in sea urchin eggs is also based on these features. LePage (1948) identified a phosphate compound in the barium-soluble, alcohol-soluble fraction of rat carcinoma as 1,2-propanediol phosphate on the basis of the phosphorus and lead content of its lead salt and its stability to hydrolysis in 1 N HCl. Against these observations, however, the experiments by Rudney (1952, 1954) and those reported here in which 96% to 98% recovery of labeled, synthetic ester was obtained in the barium-soluble, alcohol-insoluble fraction, demonstrate in a manner that seems unequivocal that this substance is not a component of the barium-soluble, alcohol-soluble fraction. With the demonstration that this ester is not in the barium-soluble, alcohol-soluble fraction, there is, at present, no evidence that propanediol phosphate exists in sea urchin eggs.

The existence of more than one compound in the fraction is further supported by the chromatographic studies which show a minimum of three components. In the related sea urchin, *Strongylocentrotus dröbachiensis,* and in the star fish, *Asterias forbesii,* Mende and Chambers (1953) found that 23% and 60%, respectively, of the phosphorus of this fraction was hydrolyzed to orthophosphate in three hours at 100° C. in 1 N HCl. Further identification or characterization of these components has not been attempted.

In several investigations of the permeability of sea urchin embryos to inorganic phosphate a greatly increased rate of penetration after fertilization has been reported, but subsequent changes in rate have not been followed beyond seven hours in *S. purpuratus* and four or five hours in *Arbacia punctulata.* In these early stages the uptake proceeds at a uniform rate. The experiments reported here show that during later cleavages and blastulation the rate of penetration continues to rise markedly, but that coincident with the onset of the first major form change, gastrulation, the rate shows an abrupt and considerable drop which continues at least to the early pluteus. It will be of interest to determine if this new pattern reflects some profound change at gastrulation either of the metabolism within the cells of the embryo, or of a surface transport mechanism for phosphate correlated with the differentiation of the ectoderm.

Summary

1. The quantity of phosphorus in the barium-soluble, alcohol-soluble fraction of the acid-soluble phosphate compounds of the embryos of the sea urchin, *Strongylocentrotus purpuratus* (Stimpson), was measured until the formation of the early pluteus stage. In two experiments the phosphorus in the fraction decreased by 18.5% and 40.7% in reaching the late prism stage (72 hours).

2. During this period, the total phosphorus of the embryos increased an average of 14.4%.

3. The rate of penetration of radioactive phosphorus from sea water into the embryos during these experiments increased very greatly during cleavage and blastulation, reached a maximum at the onset of gastrulation, and decreased subsequently to a middle level at the prism stage.

4. The rate of uptake of radioactive phosphorus into the barium-soluble, alcohol-soluble fraction was extremely low in unfertilized eggs and in fertilized eggs before the first cleavage, amounting to 0.7% to 1.4% of the total uptake.

5. The rate of uptake of radioactive phosphorus into this fraction in cleaving eggs and embryos mirrored that of the total phosphorus, and the percentage of the labeled phosphorus in the fraction was relatively constant at all stages, averaging 6.5% of the total.

6. Chromatographic examination of the fraction has indicated the existence of at least three components.

7. It is concluded that there are several components in the fraction, at least one of which is a stored material that is steadily metabolized during development, and one or more others synthesized at a rate that is governed largely by the supply of available phosphate.

8. Synthetic radioactive propanediol phosphate added to a perchloric acid homogenate of eggs separated to the extent of 97% into the barium- and alcohol-insoluble, rather than the barium-soluble, alcohol-soluble fraction. It is concluded from this, in contrast to previous reports, that the latter fraction does not contain propanediol phosphate and that no evidence remains for the existence of this ester in sea urchin eggs.

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